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Retrospective and Prospective Verification of the Cepheid Xpert Influenza Virus Assay▽

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We performed a retrospective (n = 121) and prospective (n = 305) verification of the Cepheid Xpert Flu assay to determine its performance characteristics. The overall sensitivity and specificity were 93% and 100%, respectively. Nasopharyngeal specimen sensitivities were 100% for seasonal influenza A/H1 virus and influenza A/H3 virus, 90% for influenza A/2009/H1N1 virus, and 95% for influenza B virus.

The introduction of influenza A/2009/H1N1 virus into our communities has challenged clinical laboratories to consider the most appropriate diagnostic tools for the laboratory diagnosis of influenza (4). Rapid antigen tests are limited by inferior sensitivity (2), while culture and molecular tests are constrained by longer turnaround times. The recently FDA-cleared Xpert Flu assay (Cepheid, Sunnyvale, CA) is a rapid, random-access molecular test capable of detecting and differentiating influenza A, influenza B, and influenza A/2009/H1N1 viruses from nasal wash fluid samples/aspirates and nasopharyngeal (NP) swabs. The Xpert assay allows extraction, amplification, and detection to take place within a single-use disposable cartridge.

We conducted a verification study using both retrospective respiratory samples (multiple specimen types) and prospective NP swabs to analyze the performance of the Xpert Flu assay. The reference method was defined as our laboratory-derived assay (LDA), for which 200 μl of specimen was extracted using the Total Nucleic Acid Isolation Kit on the MagNAPure (Roche Applied Science, Indianapolis, IN) with amplification and detection performed on the ABI 7500 or the ABI 7500 FAST (Life Technologies, Carlsbad, CA) using primer and probe sequences previously described for influenza A (1) and influenza B (3) viruses. The Xpert Flu assay was performed in accordance with the manufacturer's instructions for cleared specimen types (nasal wash fluid samples/aspirates and NP swabs). Noncleared specimens were tested directly from viral transport medium without preprocessing.

The retrospective study included 121 samples collected between January 2006 and December 2010 and stored at −70°C. Results were originally obtained using viral culture, xTAG RVP (Luminex Molecular Diagnostics, Austin, TX), or the LDA. All discrepant results (e.g., Xpert Flu negative, archived positive) were retested using the reference LDA. The retrospective specimens consisted of NP swabs (n = 75), bronchoalveolar lavage fluid samples/wash fluid samples (n = 33), NP aspirates (n = 4), tracheal aspirates (n = 4), sputum samples (n = 3), and nasal wash fluid samples (n = 2). Prospective specimens consisted of 305 NP swabs collected from patients presenting with influenza-like illness between December 2010 and February 2011 and tested by the reference LDA. All specimens positive for influenza A virus were typed using the Prodesse ProFAST+ assay (Gen-Probe, San Diego, CA) if a type had not already been determined by routine testing using the xTAG RVP assay. Starting in 2009, NP swabs were collected using flocked swabs transported in Universal Transport Medium (Becton Dickinson, Franklin Lakes, NJ). All other specimens were tested in viral transport medium (Remel, Lenexa, KS). This study was approved by the University of North Carolina Institutional Review Board.

Lower limits of detection (LLD) were determined using viral stocks of seasonal influenza A/H1 virus and influenza B virus quantified by and obtained from Advanced Biotechnologies (Columbia, MD) or viral stocks of influenza A/H3 virus and influenza A/2009/H1N1 virus quantified by real-time reverse transcription (RT)-PCR. Dilutions were tested in triplicate for 3 consecutive days. In-house studies had previously determined the LLD of the LDA to be 375 viral particles per ml (vpm) for influenza A/H1 virus and 860 vpm for influenza B virus. The LLD of Xpert Flu were 1,000 vpm for seasonal influenza A/H1 virus, 3,570 vpm for influenza A/H3 virus, 5,000 vpm for influenza A/2009/H1N1 virus, and 860 vpm for influenza B virus. Of note, all replicates of 3,000 vpm of influenza A/2009/H1N1 virus were detected by the 2009/H1N1 virus probe, but only 78% (7/9) were detected by the influenza A virus probe. The Xpert software reports influenza A virus probe-negative, 2009/H1N1 virus probe-positive samples as invalid.

TABLE 1. Statistical analysis of cycle threshold values of discrepant samples from the prospective study

Test result	n	Mean C_T (95% CI)[a]	P value
Influenza A virus (all)	59	22.72 (21.33, 24.11)	
Xpert positive	55	21.81 (20.62, 22.97)	<0.0001
Xpert negative	4	35.29 (33.75, 36.84)	
Influenza B virus (all)	57	23.42 (22.14, 24.70)	
Xpert positive	54	22.81 (21.67, 23.95)	<0.0001
Xpert negative	3	34.32 (31.88, 36.76)	

[a] C_T, cycle threshold; CI, confidence interval.

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TABLE 2. Summary of sensitivity data by viral type

Specimens	% Sensitivity				
	Influenza A/H1 virus	Influenza A/H3 virus	Influenza A/2009/H1 virus	Influenza B virus	All types
Retrospective					
All	100 (16/16)[a]	100 (20/20)	80 (24/30)	100 (15/15)	93 (75/81)
NP	100 (9/9)	100 (16/16)	86 (19/22)	100 (10/10)	95 (54/57)
Non-NP	100 (7/7)	100 (4/4)	63 (5/8)	100 (5/5)	88 (21/24)
Prospective (all NP)	ND[b] (0/0)	100 (13/13)	91 (42/46)	95 (54/57)	94 (109/116)
Combined					
All	100 (16/16)	100 (33/33)	87 (66/76)	96 (69/72)	93 (184/197)
NP	100 (9/9)	100 (29/29)	90 (61/68)	95 (59/62)	94 (158/168)
Non-NP	100 (7/7)	100 (4/4)	63 (5/8)	100 (10/10)	90 (26/29)

[a] The values in parentheses are ratios of Xpert-positive to LDA-positive specimens.
[b] ND, none detected.

Analytical specificity was determined using high-concentration stocks of: *Staphylococcus aureus* (methicillin susceptible and methicillin resistant), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, viridans group streptococci, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Bordetella pertussis*, *Bordetella parapertussis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, oropharyngeal flora, nasal flora, adenovirus, metapneumovirus, respiratory syncytial viruses A and B, rhinovirus, enterovirus, and parainfluenza viruses 1, 2, and 3. No cross-reactivity was observed.

The retrospective verification included a broad spectrum of respiratory samples (see above), of which 82 were positive for seasonal influenza A/H1 virus ($n = 16$), influenza A/H3 virus ($n = 20$), influenza A/2009/H1N1 virus ($n = 31$), or influenza B virus ($n = 15$). Thirty-nine negative samples were also tested. Sensitivity was 100% for seasonal influenza A/H1 virus, influenza A/H3 virus, and influenza B virus. However, only 24 (77%) of 31 influenza A/2009/H1N1 virus-positive samples were detected by the Xpert Flu assay. One of these discrepant specimens was excluded, as it was not positive upon repeat testing with the LDA; thus, the sensitivity for influenza A/2009/H1N1 virus was 80% (24/30). The false-negative specimens included NP swabs ($n = 3$), tracheal aspirates ($n = 2$), and a bronchoalveolar lavage fluid sample ($n = 1$). Specificity was 100%.

Prospective NP swabs were tested by Xpert Flu upon receipt. Residual patient specimen was kept at 4°C until additional testing was complete (LDA and Prodesse ProFAST+) (median, 5 days; range, 0 to 24 days). Of the 305 NP swabs, 116 were positive by the reference LDA for influenza A virus ($n = 59$) and influenza B virus ($n = 57$). The 59 influenza A virus-positive samples were typed using the Prodesse ProFAST+ assay, and 46 (78%) contained influenza A/2009/H1N1 virus and 13 (22%) contained influenza A/H3 virus. Seven samples produced results that were discrepant between the two assays in the prospective study. The Xpert Flu assay detected 55/59 (93%) influenza A virus-positive swabs and 54/57 (95%) influenza B virus-positive swabs. The four influenza A virus-positive NP specimens missed by Xpert Flu were all detected and typed as influenza A/2009/H1N1 virus by the Prodesse ProFAST+ assay. Specimens detected solely by the LDA had significantly higher crossing threshold values than those positive by both tests (Table 1). Thus, Xpert Flu false-negative results are likely due to decreased analytical sensitivity relative to the reference LDA. The Xpert Flu assay was 100% specific.

A summary of the sensitivity data for the verification study described here can be found in Table 2. There was no statistically significant difference between the combined sensitivity of NP specimens and non-NP specimens ($P = 0.15$), presumably due to the low number of positive non-NP specimens. Our data show the feasibility of testing non-NP specimens by the Xpert Flu assay, though more studies are needed to determine if there is a difference in sensitivity. Interestingly, no invalid results were obtained due to the lack of amplification of internal control, indicating that no inhibition was observed in any specimen type. Specificity was consistently 100% among all specimen types. Although the LDA is more sensitive than the Xpert Flu assay for influenza A/2009/H1N1 virus and influenza B virus, it is a traditional real-time RT-PCR assay that requires extraction and batch processing. During influenza season, we perform the LDA three or four times a day, achieving a turnaround time of 8 to 24 h. This is an unacceptable time to result, particularly for our emergency department patients. The Xpert Flu assay has a 2-min hands-on time and a 76-min run time. It is random access and simple to perform and provides clear results, allowing laboratory personnel not proficient in molecular techniques to perform the test. The combined ease of use and acceptable sensitivity of the Xpert Flu assay make it an attractive approach to the rapid molecular diagnosis of influenza.

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